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# Chemometric classification of new *Olea europaea* L. cultivars developed through a crossbreeding program in Tunisia

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## ABSTRACT

Five new olive (*Olea europaea* L.) cultivars issued from a Tunisian breeding program were released in 2017. This program aimed to improve the oil quality of the local cultivar 'Chemlali Sfax' which had mainly low oleic acid content. A wide genetic diversity was observed within the new cultivars which differ from the typical cultivar. The results of the morphological evaluation of the leaf, fruit and stone showed mainly a significant increase of the fruit size (medium) and the appearance of new morphological states for the fruit and the stone.

**KEYWORDS:** Chemlali sfax, hybridization, morphology

## INTRODUCTION

Olive genetic improvement by the hybridization method has been adopted in several countries such as Israel [1], Spain [2], Italy [3] and Morocco [4]. The most significant results come from Israel, where several varieties have been characterized and released. Several new varieties have been obtained: Kadesh [5], Barnea [6], Maalot [7], Askal [8] and Kadeshon, Sepoka and Masepo [9]. In Spain, a hybridization program has been carried out since 1991. Reciprocal crosses between cultivars have been carried out with the aim of reducing the juvenile period and improving olive production and oil yield. Recently, a new variety (Chiquitita) was selected and released in Spain [10].

Following the study of Fontanazza and Baldoni [11], a genetic improvement project by controlled crosses was initiated in 1993 and concerned some Mediterranean countries. In Tunisia, this program has interested the Chemlali Sfax variety to improve the acidic composition of its oil. Indeed, Chemlali Sfax has low oleic acid (53 to 56%) and high palmitic acid (17 to 21%) according to Grati-Kamoun and Khlif [12] and Zarrouk *et al.* [13]. The hybridization program generated a collection of hybrids using the Chemlali Sfax variety and other Tunisian and foreign varieties [14]. The obtained seedlings were planted in 1997 at the experimental farm of the Olive Tree Institute in Sfax region. Initial studies are interested in screening progenies for chemical composition. Since 2000, a preliminary selection based on the acid composition has been carried out [15] and the selected hybrids have been planted in a comparative trial in the Sfax region in 2005.

In addition, other previous studies have been carried out on olive tree seedlings issued by the Tunisian breeding program whose purpose is to study and evaluate the distribution of their genetic diversity.

Barranco *et al.* [16] have proposed a guide for morphological description using different organs such as tree, leaf, fruit and stone. The studies on the morphological characterization of olive hybrids of the chemlali sfax variety have shown great genetic variability and most of the offspring within the crossings observed very significant differences [17]. Previous studies conducted on olive seedlings 'Chemlali sfax' showed a great variability between morpho-agronomic [18] and architectural characters [19].

Recently, five new cultivars obtained in the Tunisian crossbreeding program were proposed for release, accepted in 2016, and published in the Official Journal of Republic of Tunisia [20].

The objective of this work was to describe the variability observed for the main morphological characters in the five

released cultivars planted in the comparative field trial established in 2005.

## MATERIAL AND METHODS

The study was carried out on olive trees from 5 seedlings of Chemlali Sfax'. In details, the descendants were obtained from self pollinated Chemlali Sfax (Chemlali Mhassen), from crosspollination 'Chemlali Sfax with Chemchali Gafsa (Zeitoun Allyssa and Zeitoun Ennour) and Lucques (Zeitoun Ennwader), while the cross of Janouby Mwarref was not identified (Table 1). Seedlings were planted in a comparative field trial during 2005-2006 with a density of 416 trees $ha^{-1}$ (4m x 6m) in the Research Station of Taous, which is about 26 km far from Sfax (34° 56 North, 10° 36 East). Sfax region is located in the center part of Tunisia with arid climate and sandy and highly permeable soils.

Morphological description was carried out according to the primary characterization of olive varieties cited by the International Olive Council [21] by using 21 characters of the leaf, fruit and stone.

The morphological characterization was performed on 40 fruits and their stones and 40 mature leaves in November (three trees for each sample) during three years (2013-2015). Concerning the distribution of qualitative characteristics, we used the average of the three replications for each year then the average of the three years for each character to describe the morphology of the different parts for each hybrid.

The morphological follow-up included both quantitative and qualitative variables (Table 2). For leaf, the shape (LS) (length (LL)/ width (LWI) ratio) were determined. For fruit, the weight (FW), Shape (FS) (length (FL)/width (FWI) ratio), Symmetry (FSy), Position of the maximum transverse diameter (FPDM), Apex (FA), Base (FB), lenticels (FLe), Nipple (FN), location of start color change and maturity color were determined. For stone, we have noted the weight (SW), Shape (SS) (length (SL)/width (SWI) ratio) Symmetry (SSy), Position of the maximum transverse diameter (SPDM), Apex (SA), Base (SB), Surface (SSu), distribution of groves (SG) and termination of the apex. Morphological description was compared to that of Chemlali Sfax reported in literature.

### Data analysis

The global data set is constituted by the global olive data set (600×8 olive variables) and the correspondent global stone data set (600×9 stone variables). Experimental data were assembled into a 600 samples (40 samples per tree per year; 40 sample * 5 hybrids * 3 year) LDA calculations were done on raw data using SPSS 23.0 for Windows (SPSS Inc., Chicago, USA).

For each hybrid the data of the means of three year for morphological parameter, cluster analysis was conducted on the squared Euclidean Distance matrix with the Unweighted Pair Group method based on Arithmetic Averages (UPGMA). The same data were used to perform principal component analysis. These analyses were undertaken by using the XLSTAT 2014.5.03.

## RESULTS

### Morphological Description

The quantitative parameters for the leaf, fruit and stone were described in Tables 2-4. While, the qualitative parameters were described in Table 5.

From the analysis of the continuous variables (Tables 2-4), JM has the highest value for seven parameter, length of the leaf and the fruit, width of the fruit and stone, weight of the fruit and stone and of the shape leaf. The weights of the stone of the other hybrids are no marked differences are observed (from 0.31 g to 0.34 g).

Morphological description data were reported in Table 5. Leaves were mostly with elliptic-lanceolate shape and flat longitudinal curvature. All descendants had fruit with a central maximum diameter, a truncate base, many lenticels, black maturity color and without nipple. Nevertheless, the fruit weight, shape and apex were respectively low to medium, ovoid to elongated and pointed to rounded.

All stones of the selected cultivars were asymmetric, with pointed apex and rounded base (except JM with pointed base) and regular

Table 1: Names, codes and crosses of new released cultivars in Tunisia

| Name | Abbreviation | Cross |
|---|---|---|
| Janouby Mwarref | JM | Unknown |
| Chemlali Mhassen | CM | Chemlali Sfax self pollinated |
| Zeitoun Allyssa | ZA | Chemlali Sfax * Chemchali Gafsa |
| Zeitoun Ennwader | ZEW | Chemlali Sfax * Lucques |
| Zeitoun Ennour | ZEN | Chemlali Sfax * Chemchali Gafsa |

Table 2: Average of the leaf continuous data variable

| Variable | JM | CM | ZA | ZEW | ZEN |
|---|---|---|---|---|---|
| Length (cm) | 6,09±0,40 | 5,10±0,35 | 5,66±0,05 | 4,98±0,16 | 5,53±0,03 |
| Width (cm) | 0,99±0,04 | 1,13±0,05 | 1,19±0,07 | 1,07±0,01 | 1,05±0,06 |
| Shape | 6,22±0,59 | 4,55±0,10 | 4,81±0,25 | 4,65±0,11 | 5,34±0,34 |

Table 3: Average of the fruit continuous data variable

| Variable | JM | CM | ZA | ZEW | ZEN |
|---|---|---|---|---|---|
| Weight (g) | 2,97±0,45 | 2,21±0,10 | 1,53±0,06 | 1,67±0,26 | 2,14±0,66 |
| Length (cm) | 2,14±0,12 | 1,78±0,01 | 1,77±0,04 | 1,81±0,13 | 1,80±0,12 |
| Width (cm) | 1,52±0,08 | 1,36±0,04 | 1,11±0,08 | 1,18±0,11 | 1,26±0,11 |
| Shape | 1,41±0,02 | 1,31±0,05 | 1,61±0,15 | 1,55±0,03 | 1,43±0,05 |

Table 4: Average of the stone continuous data variable

| Variable | JM | CM | ZA | ZEW | ZEN |
|---|---|---|---|---|---|
| Weight (g) | 0,39±0,02 | 0,33±0,04 | 0,34±0,03 | 0,31±0,03 | 0,34±0,05 |
| Length (cm) | 1,38±0,04 | 1,25±0,06 | 1,41±0,04 | 1,31±0,04 | 1,33±0,11 |
| Width (cm) | 0,67±0,01 | 0,64±0,03 | 0,56±0,03 | 0,57±0,03 | 0,61±0,03 |
| Shape | 2,07±0,10 | 1,98±0,05 | 2,52±0,21 | 2,31±0,06 | 2,19±0,12 |

distribution of groves (Table 5). Most of them were characterized by medium weight, elliptic shape and apex with mucro.

On the basis on these data, we noted that each new cultivar can be distinguished by a particular character:

- Cultivar Janouby Mwarref had lanceolate leaf shape, pointed base and scabrous surface.
- Cultivar Chemlali Mhassen had both position of maximum transverse diameter towards apex and rugose surface of the endocarp.
- Cultivar Zeitoun Allyssa had elongated endocarp shape.
- Cultivar Zeitoun Ennwader was mainly characterized by smooth surface.
- Cultivar Zeitoun Ennour had apex termination without mucro.

## Statistics Analysis

*Linear discriminant analysis of the olive and stone data*

The purpose of this study was to assess whether data sets for olives and/or stone contain sufficient information to allow the development of linear discrimination rules between the five hybrids. Figures 1 and 2 show the LDA plot of olives and stone data. Consequently, the LDA classification results presented in Table 6 show the percentage of expected group membership of the original samples. In accordance with the olive fruit, the percentages of correct classifications reaches 83.3% for the JM, 79.2% for ZEW, 73.3% for CM, 59.2% for ZA and 55.8% for ZEN. Whereas, in conformity with the stone variable data set the correct classification reaches 85% for the JM, 90% for ZEW, 95% for CM, 77.5% for ZA and 65.8% for ZEN. According to these results, the stone variable data are better desciptors of all hybrids.

Nonetheless, a detailed analysis of Figure 1 shows that there is some overlap of the samples according to the olive parameters whereas according to the Figure 2 of the distribution of the hybrids according to the stone parameters shows that the zones corresponding to each cultivar are well resolved.

In addition, Wilks' Lambda and F tests were used to evaluate the discriminant capacity of the variables (Table 7). According to Paula *et al.* [22], the discrimination capacity of the variable is high when its Wilks's Lambda value is low and the F test is high. Therefore, the result of Wilks' Lambda and F-tests (Table 7) shows that the leaf can contribute only with the shape parameter, which shows that leaf parameters do not vary between hybrids. For the olive, four most discriminate variables are length, width, ratio length/width and the apex and the five most discriminating stone variables are width, the ratio length/width, Shape, PDM and surface.

## Principal Components Analysis (PCA)

The principal component analysis (PCA) revealed two major components totaling 84.8% of the total variance (57.99% and 26.81% respectively) (Figure 3). The most important contribution in the first component is performed positively by the weight, width of fruit and stone (FW, FWI, SW and SWI),

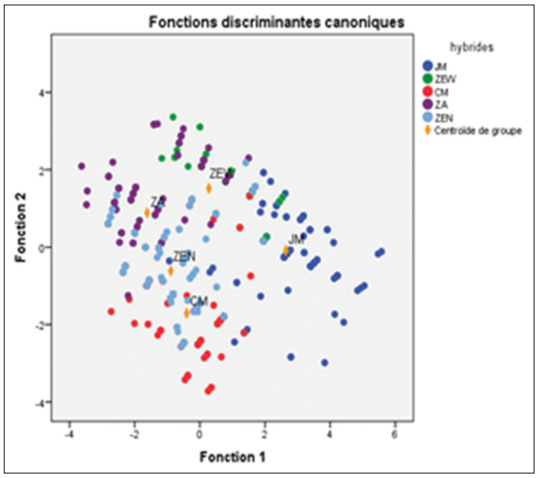


**Figure 1:** Linear discriminant plots of the olive data set

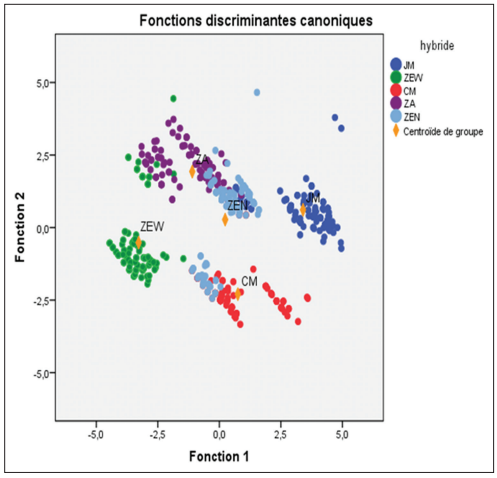


**Figure 2:** Linear discriminant plots of the stone data set

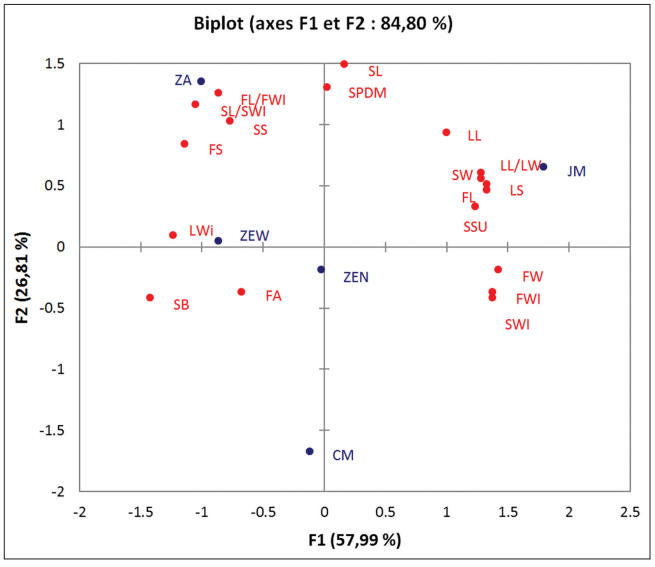


**Figure 3:** Principal components analysis based on the morphologic characteristics

Table 5: Morphological traits of leaf, fruit and endocarp evaluated for five olive cultivars issued from seedlings of 'Chemlali Sfax'

| Organ | Character | JM Hd1128 | CM Hd1321 | ZA Hd81 | ZEW Hd135 | ZEN Hd166 |
|---|---|---|---|---|---|---|
| Leaf | Shape | Lanceolate | Elliptic-Lanceolate | Elliptic-Lanceolate | Elliptic-Lanceolate | Elliptic-Lanceolate |
| | Longitudinal curvature | Flat | Flat | Flat | Flat | Flat |
| Fruit | Weight | Medium | Medium | Low | Low | Medium |
| | Shape | Ovoid | Ovoid | Elongated | Elongated | Ovoid |
| | Symmetry | Asymmetric | Asymmetric | Asymmetric | Asymmetric | Asymmetric |
| | PDM | Central | Central | Central | Central | Central |
| | Apex | Pointed | Rounded | Rounded | Pointed | Rounded |
| | Base | Truncate | Truncate | Truncate | Truncate | Truncate |
| | Nipple | Absent | Absent | Absent | Absent | Absent |
| | Color change | From the apex | From the apex | From the apex | From the apex | Uniform |
| | Lenticels | Many | Many | Many | Many | Many |
| | Maturity color | Black | Black | Black | Black | Black |
| Endocarp | Weight | Medium | Medium | Medium | Medium | Medium |
| | Shape | Elliptic | Elliptic | Elongated | Elliptic | Elliptic |
| | Symmetry | Asymmetric | Asymmetric | Asymmetric | Asymmetric | Asymmetric |
| | PDM | Central | Towards apex | Central | Towards apex | Central |
| | Apex | Pointed | Pointed | Pointed | Pointed | Pointed |
| | Base | Pointed | Rounded | Rounded | Rounded | Rounded |
| | Surface | Scabrous | Rugose | Rugose | Smooth | Rugose |
| | distribution of groves | Regular | Regular | Regular | Regular | Regular |
| | Apex termination | With mucro | With mucro | With mucro | With mucro | without mucro |

PDM: Position of maximum transverse diameter

also by leaf size (LL, LL/LWI and LS), fruit length (FL) and stone surface (SSu). The PC1 was also correlated negatively with stone base (SB), width leaf (LWI) and fruit shape (FS).

The second component is positively correlated with position of the maximum transverse diameter of stone (SPDM); shape stone (SS), length stone (SL) and the ratio length/width of fruit (FL/FWI) and stone (SL/SWI).

In fact, CP1 clearly separates the hybrids by the weight of fruit and stone from the lowest olive weight to the highest, also by the stone and fruit width from the lowest to the highest and by leaf parameters. In accordance with CP1 the hybrids are classed in 3 groups the first one contains ZEW and ZA characterize by the lowest value of FW, SW, FWI and SWI. The second contains ZEN and CM; finally the third contains only by the JM characterized by the high value of parameters mentioned before. While CP2 separates the hybrids according to their parameters quantitative of length stone and length/width ratio of fruit and stone from the lowest value of highest value. In accordance with CP2 the hybrids are classed in 3 groups, the first one contains only the CM characterized by the lowest of parameters and position of the maximum diameter of stone in Apex. The second contains ZEN, ZEW and JM; finally the third contains only by the ZA characterized by the highest value of parameters and elongated stone.

### Hierarchical Cluster Analysis (HCA)

The dendrogramme resulting from the cluster analysis of the morphological parameters is shown in Figure 4.

The first group is composed of two progenies obtained from crossbreeding of Chemlali × Chemchali (ZA) and the progenies from Chemlali × Lucques (ZEW), these hybrids are characterized by a very low value of fruit and stone size. The second group contains the Chemlali × Chemchali progenies (ZEN) and the progenies obtained from crossbreeding of Chemlali × Chemlali (CM), which are characterized by the lowest value of length/width ratio of fruit and stone. The last group is constituted from JM progenies which has characterized by the highest for stone and fruit weight, it is also the only hybrid with lanceolate leaf.

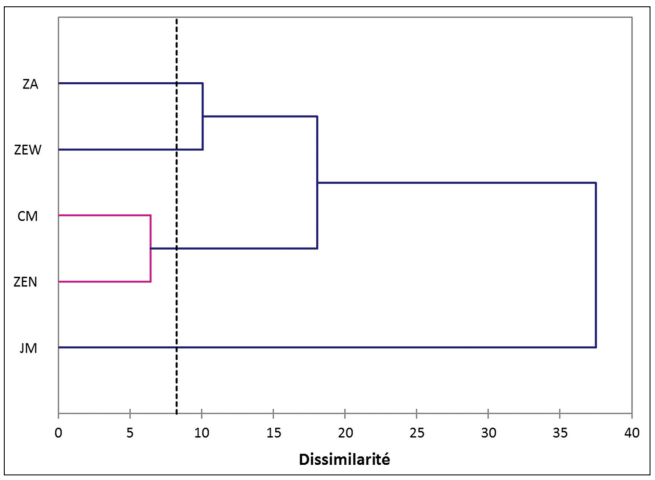


**Figure 4:** UPGMA dendrogram of the hybrids based on the morphologic characteristics

## DISCUSSION

Morphological characterization of the original cultivar Chemlali Sfax was reported by Trigui and Msallem [23] and Barranco *et al.* [16]. In comparison with these studies, the selected cultivars were similar to Chemlali Sfax for leaf longitudinal curvature (flat), position of maximum transverse diameter

Table 6: Classification results of the test set (percentages of correct classifications)

| Data set used in classification | Hybrids | Hybrids | | | | |
|---|---|---|---|---|---|---|
| | | JM | CM | ZA | ZEW | ZEN |
| Olive | JM | 83,3 | 6,7 | 0 | 6,7 | 3,3 |
| | CM | 0,8 | 73,3 | 0 | 2,5 | 23,3 |
| | ZA | 0 | 0 | 59,2 | 12,5 | 28,3 |
| | ZEW | 5 | 8 | 6,7 | 79,2 | 8,3 |
| | ZEN | 0,8 | 22,5 | 14,2 | 6,7 | 55,8 |
| Stone | JM | 85 | 0 | 0 | 0 | 15 |
| | CM | 1,7 | 95 | 0 | 0 | 3.3 |
| | ZA | 0 | 0 | 77.5 | 0 | 22,5 |
| | ZEW | 0 | 0 | 9.2 | 90 | 0.8 |
| | ZEN | 0,8 | 24.2 | 9.2 | 0 | 65,8 |

Tableau 7: Wilks' Lambda and F-tests of group means

| Variable | Lambda de Wilks | Test-F | Variable | Lambda de Wilks | Test-F |
|---|---|---|---|---|---|
| Feuille | | | Stone | | |
| LL | 0.65 | 74.763 | SL | 0.842 | 27.973 |
| LWI | 0.661 | 71.246 | SWI | 0.652 | 79.451 |
| LL/LWI | 0.475 | 153.661 | SL/SWI | 0.614 | 93.451 |
| LS | 0.65 | 74.627 | SS | 0.697 | 64.650 |
| Fruit | | | | | |
| FL | 0.559 | 117.50 | SSym | 0.914 | 13.93 |
| FWI | 0.387 | 235.395 | SA | 0.962 | 5.831 |
| FL/FWI | 0.583 | 106.282 | SB | 0.792 | 39.112 |
| FS | 0.653 | 78.938 | SPDM | 0.414 | 210.82 |
| FSym | 0.846 | 27.126 | SSu | 0.237 | 478.964 |
| FA | 0.441 | 188.765 | Apex termination | 0,855 | 22,345 |
| FB | 0.973 | 4.175 | | | |
| FPDM | 0.981 | 2.873 | | | |

(central), base shape (truncate) and nipple (absent) of the fruit and stone shape (elliptic). The low number of characters (5) similar to the original variety showed a high genetic variability in these seedlings, as reported by Laaribi *et al.* [17] and Guellaoui *et al.* [24].

In fact, all the selected cultivars showed new morphological states of leaf, fruit and stone in comparison with the typical of 'Chemlali Sfax' cultivar. We revealed the appearance in seedlings of lanceolate leaf shape, medium weight, elongated, asymmetric and pointed apex for the fruit. For the stone, most of the characters of the cultivars showed new states (medium weight, elongate shape, asymmetric, maximum diameter towards apex, pointed apex, rounded base, rugose and scabrous surface and apex without mucro). These differences in morphological characters of the new cultivars were due mainly to genetic variation, as all seedlings were grown in the same agro-climatic conditions, as cited by Manai *et al.* [25]. Even, morphological differences were noted for two hybrids derived from the same cross (Chemlali Sfax x Chemchali Gafsa).Thus, Zeitoun Allyssa and Zeitoun Ennour were different for five morphological characters, in accordance with Bartoloni and al. [31].

Enhanced fruit size was noted for the new cultivars in comparison to the 'Chemlali Sfax', which had low, fruit weight, 1 g as reported by Grati-Kamoun and Khlif [12]. In fact, most of the studied seedlings presented medium fruit weight (2 to 4 g according to IOC [21]). This can be considered as an important criterion of the improvement of olive oil content, in accordance with Bellini [3], the increase in olive weight improves the olive oil content. Also, according to other authors [13], it is important to study the fresh average weight of the fruit for the new cultivars if it is for oil or table or even both uses. The higher fruit weight of cultivars Zeitoun Allyssa, Zeitoun Ennwader and Zeitoun Ennour came from the parental genitors Chemchali Gafsa and Lucques, which had fruit weight respectively 1.8 to 2.8 g [12] and 2 to 4 g [16]. As revealed by many authors [1, 3, 26-31], hybridization is an important method to increase the genetic variability in olive fallowing the selection of new interesting genotypes.

## CONCLUSION

This present study proves the interesting genetic diversity of the studied new cultivars in the morphological characters. It allowed us to depict five released olive cultivars that showed specific fruit and stone characters when compared to Chemlali Sfax.

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## REFERENCES

1. Lavee S. Aims, methods, and advances in breeding of new olive (*Olea europaea* L.) cultivars. Acta Horticulturae. 1990;286:23-36.
2. Rallo L. Selection and breeding of olive in Spain. Olivae. 1995;59:46–53.
3. Bellini E. Variabilità genetica ed ereditarietà di alcuni caratteri in semenzali d'incrocio di olivo. Olivae. 1993;49:21-34.
4. Charafi J, Rahioui B, El Meziane A, Moukhli A, Boulouha B, Modafar CE and Khadari B. Verifying the reliability of hybrid issued from the cross ''Picholine marocaine clones X Picholine du Languedoc''. African Journal of Biotechnology. 2007;6(24):2776-2779.
5. Lavee S. Kadesh table olive. HortScience. 1978;131(1):62-63.
6. Lavee S, Haskal A and Wodner M. " Barnea " A new olive cultivar from first breeding generation. Olea. 1986;17: 95-99.
7. Lavee S, Harshemesh H, Haskal A, Meni Y, Wodner M, Ogrodovich A, Avidan B, Wiesman Z, Avidan N and Trapero-Casus A. 'Maalot' a new cultivar for oil extraction resistant to *spilocaea oleagina* (cast.). Olivae. 1999;78:51-59.
8. Lavee S, Avidan B and Meni Y. "Askal", a New High Performing Oil Variety for Intensive and Supper-Intensive Olive Orchards. Olivae. 2003;97:53-59.
9. Lavee S, Avidan B, Meni Y, Haskal A and Wodner M. Three new semi-dwarf varieties of olive tree for table use. Olivae. 2004;102:33-41.
10. Rallo L, Barranco D, De La Rosa L and Leon L. Chiquitita olive. HortScience. 2008;43:529-531.
11. Fontanazza G and Baldoni L. Proposed programme for the genetic improvement of the olive. Olivae. 1990;34:32-40.
12. Grati-Kamoun N and Khlif M. Caractérisation technologique des variétés d'olivier cultivées en Tunisie. Revue Ezzitouna (numéro spécial). 2001;69.
13. Zarrouk W, Baccouri B, Taamalli W, Trigui A, Daoud D and Zarrouk M. Oil fatty acid composition of eighteen Mediterranean olive varieties cultivated under the arid conditions of Boughrara (southern Tunisia). Grasas y aceites. 2009;60(5):498-506.

14. Trigui A. Improving the quantity and quality of olive production in Tunisia: unavoidable need and outlook for olive identification and breeding. Olivae. 1996;61:34-40.
15. Ben Amar F, Mezghani-Aiachi M, Yengui A, Belguith H, Harrab S and Hergli MK. Variability in the agronomic performance of a collection of olive hybrids (*Olea europaea* L.) of the local 'Chemlali Sfax' oil-olive variety. Olivae. 2015;122:16-21.
16. Barranco D, Cimato A, Fiorino P, Rallo L, Touzani A, Castaneda C, Serafini F and Trujillo I. World Catalogue of Olive Varieties. International Olive Oil Council, Madrid, Spain; 2000.
17. Laaribi I, Meaghani-Aiachi M and Mars M. Phenotypic diversity of some olive tree progenies issued from a Tunisian breeding program. European Scientific Journal. 2014;10(6):292-313.
18. Trigui A, Yengui A and Belguith H. Olive germplasm in Tunisia.Olea. 2006;25:19-23.
19. Aïachi M and Trigui A. Analyse de l'architecture des descendances issues de croisements dirigés de la Chemlali de Sfax et d'autres variétés d'olivier (*Olea europaea*. L). Revue Ezzaitouna. 2001;6(1 et 2) 33-58.
20. JORT. Official Journal of Republic of Tunisia, Year 160 n° 33, April 25th 2017. p 1318.
21. IOC. Méthodologie pour la caractérisation des variétés d'oliviers. Projet sur la conservation, caractérisation, collecte et utilisation des ressources génétiques de l'olivier. Communauté Européenne. Conseil Oléicole International, 1997; ISSN 0255-9978.
22. Paula B.M, Pinheiro, Joaquim C.G, Esteves da Sila. Chemometric classification of olives from three Portuguese cultivars of *Olea europaea* L. Analytica Chimica Acta. 2005;544:229-235.
23. Trigui A and Msallem M. Catalogue des variétés Autochtones et types locaux; 2002.
24. Guellaoui I, Ben Amar F, Boubaker M and Yangui A. caracterisation phenotypique d'hybrides d'olivier (*Olea europaea* L) issus de la variete locale " CHEMLALI SFAX " Revue des BioRessoources. 2015;38-53.
25. Manaï H, Mahjoub-Haddada F, Oueslati I, Daoud D and Zarrouk M. Characterization of monovarietal virgin olive oils from six crossing varieties. Scientia Horticulturae. 2008;115:252–260.
26. Bartolini S, Andreini L, Guerriero R and Gentili M. Improvement of the quality of table olives in Tuscany through cross-breeding and selection: preliminary results of Leccino x Konservolia hybrids. In: Proceedings of the Second International Seminar Olivebioteq 2006- November 5th-10th. Mazara del Vallo, Marsala (Italy). 2006;1:143-146.
27. Fontanazza G, Vergari G, Patumi M and Giorio G. Preliminary results of the evaluation of yield components in an F1 segregant population of olive seedlings from the cross (Leccino x Kalamata). Acta Horticulturae. 1999;474:97-102.
28. Leon L, De la Rosa R, Barranco D and Rallo L. Ten years of olive breeding in Cordoba (Spain). Acta Horticulturae. 2004; 663:747–750.
29. León L, De La Rosa R, Barranco D, Rallo L. Agronomic characterization of 15 selections of then olive crossbreeding program of Cordoba, Spain. In: Proceedings of the Second International Seminar Olivebioteq 2006 - November 5th-10th. Mazara del Vallo, Marsala (Italy). 2006;1:87-93.
30. Pannelli G, Rosati A, Pandolfi S, Padula G, Mennone C, Giordani E and Bellini E. Field evaluation of olive selections derived from a breeding program. In: Proceedings of the Second International Seminar Olivebioteq 2006- November 5th-10th. Mazara del Vallo, Marsala (Italy), 2006;1:95-102.
31. Padula G, Giordani E, Bellini E, Rosati A, Pandolfi S, Paoletti A, Pannelli G, Ripa V, De Rose F, Perri E, Buccoliero A and Mennone C. Field evaluation of new olive (*Olea europaea* L.) selections and effects of genotype and environment on productivity and fruit characteristics. Advances in Horticultural Sciences. 2008;22(2):87-94.